REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-00-

0797

1. AGENCY USE ONLY (*Leave Blank*)	2. REPORT DATE	3. REPORT TYPE AND DATES
	29 Nov 00	Final Technical Report, 09/01/97-08/31/00

4. TITLE AND SUBTITLE
AASERT-97 Reconstitution of the 2-Nitrotoluene Oxygenase Component From its Individual α and β Subunits

5. FUNDING NUMBERS
F49620-97-1-0402

6. AUTHORS
Gibson, David T.
Lessner, Daniel J.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Microbiology

The University of Iowa
Iowa City, IA 52242

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NL
110 Duncan Avenue, Room B115
Bolling AFB DC 20332-8050

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

20010109 042

12a. DISTRIBUTION / AVAILABILITY STATEMENT

APPROVED FOR PUBLIC RELEASE: DISTRIBUTION UNLIMITED

12b. DISTRIBUTION CODE

13. ABSTRACT (*Maximum 200 words*)

Funded studies focused on the biodegradation of nitrobenzene by *Comamonas* sp. strain JS765, which was isolated in Dr. Jim C. Spain's laboratory, Tyndall AFB. The genes encoding the nitrobenzene dioxygenase system were cloned and sequenced from JS765. The nitrobenzene dioxygenase enzyme system shares high amino acid homology with other identified nitroarene dioxygenase enzymes, in particular the 2-nitrotoluene dioxygenase system from *Pseudomonas* sp. strain JS42. Nitrobenzene dioxygenase was purified to near homogeneity and had characteristics typical of dioxygenase enzymes. The substrate specificity of nitrobenzene dioxygenase was examined by conducting biotransformations. Based on the substrates examined, it appeared that the substrate specificity of nitrobenzene dioxygenase is different from related enzymes. Gene reporter technology was used to examine the transcriptional regulation of nitrobenzene and 2-nitrotoluene dioxygenase from JS765 and JS42, respectively. Results indicate that both dioxygenases are induced in the presence of salicylate and the respective nitroaromatic compound.

14. SUBJECT TERMS
Nitrobenzene, biodegradation, *Comamonas*, dioxygenase, 2-nitrotoluene, transcriptional regulation

15. NUMBER OF PAGES
12

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
unclassified	unclassified	unclassified	

Studies on Nitrobenzene Metabolism by a *Comamonas* sp. Strain JS765[1]

Daniel J. Lessner and David T. Gibson

Department of Microbiology, The University of Iowa, Iowa City, IA 52242

Introduction

Nitroaromatic compounds are very seldom produced by biosynthesis. However, there is an increasingly large amount of nitroaromatic compounds present in the environment due to industrial processes. Nitroaromatics are used as solvents and in a variety of processes such as the production of pigments and munitions (2). Due to improper storage, use, and disposal, nitroaromatic compounds are widespread pollutants of the environment. As a result, nitrobenzene , and 2,4-and 2,6-dintrotoluene have been included on the U.S. Environmental Protection Agency's list of priority pollutants (4).

Biodegradation of aromatic compounds by aerobic bacteria usually begins with the initial oxidation of the compound by a dioxygenase enzyme, which catalyzes the incorporation of both atoms of molecular oxygen into the substrate. Only recently have aerobic bacteria been isolated that utilize nitroaromatic compounds as growth substrates (21, 22). One example is *Comamonas* sp. strain JS765, which can grow on nitrobenzene as the sole source of carbon, nitrogen, and energy. Previous experiments demonstrated that JS765 uses an oxidative pathway for the degradation of nitrobenzene, with the initial reaction catalyzed by a nitrobenzene dioxygenase enzyme system (7). Other nitroaromatic dioxygenase enzyme systems have been cloned and sequenced from aerobic bacteria, these include the 2-nitrotoluene dioxygenase enzyme system (2NTDOS) from *Pseudomonas* sp. strain JS42 and the 2,4-dinitrotoluene dioxygenase enzyme system (DNTDOS) from *Burkholderia* sp. strain DNT (1, 6). However, strains JS42 and DNT are unable to grow using nitrobenzene as the carbon and energy source. The major objective of this research was to identify and characterize the nitrobenzene dioxygenase enzyme system (NBDOS) genes from *Comamonas* sp. strain JS765 with the objective of providing further insight into the structure/function relationship of dioxygenase enzymes and the evolution of nitroaromatic dioxygenases.

[1] The initial title of the AASERT-97 proposal was, 'Reconstitution of the 2-Nitrotoluene Oxygenase Component From its Individual α and β Subunits.' This work did yield useful data and Mr. Daniel J. Lessner's project was changed to 'Studies on Nitrobenzene Metabolism by a *Comamonas* sp. Strain JS765.' This change in project was communicated to AFOSR in 1999.

1) Cloning of the Nitrobenzene dioxygenase enzyme system (NBDOS) genes from *Comamonas* sp. Strain JS765.

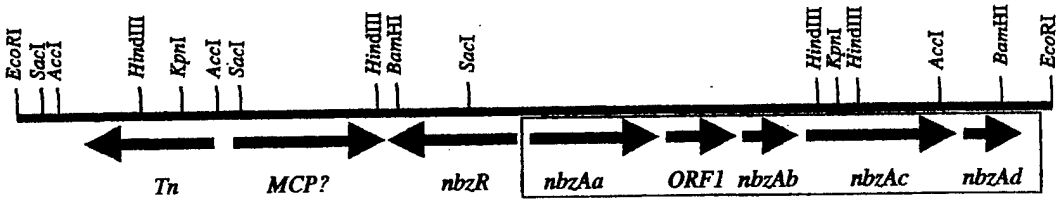
Summary. The ability of the *E. coli* strain carrying the cloned nitrobenzene dioxygenase enzyme system to oxidize nitrobenzene to catechol with concomitant release of nitrite indicates that all the of the genes necessary to encode NBDOS activity were successfully cloned. The genes are located on an 8881-bp *Eco*RI DNA fragment from *Comamonas* sp. strain JS765. Sequencing revealed eight open reading frames (Orfs). Four Orfs have high amino acid sequence homology to Orfs from Rieske non-heme iron dioxygenases, in particular 2-nitrotoluene dioxygenase from *Pseudomonas* sp. strain JS42 (9). Results of this study are in preparation to be published.

Cloning of the *nbz* genes. A DNA probe believed to be a fragment of the α-subunit of NBDO was generated by PCR using primers based on conserved sequences of known dioxygenase enzymes (NBDO probe produced by Glenn Johnson, Tyndall Air Force Base, Florida). Southern hybridization experiments demonstrated that the NBDO probe strongly hybridized to a ~8kb-*Eco*RI fragment of JS765 chromosomal DNA. A partial genomic library was constructed using gel purified *Eco*RI-digested JS765 chromosomal DNA approximately 7-10 kb in size ligated into *Eco*RI-digested pUC18 (24). Competent *Escherichia coli* DH5α (GIBCO-BRL laboratories, Gaithersburg, MD) cells were transformed with the gene library and plated onto Luria agar supplemented with 150 μg/ml ampicillin. One recombinant, designated DH5α(pDTG925) demonstrated strong hybridization to the NBDO specific probe. Whole cell biotransformation assays with DH5α(pDTG925) demonstrated that the strain was capable of catalyzing the oxidation of nitrobenzene to catechol, suggesting that all the genes (*nbz*) required for NBDOS activity were present on the pDTG925 plasmid.

Sequence analysis of pDTG925. DNA sequencing was performed by the University of Iowa DNA sequencing facility. DNA sequence analysis of pDTG925 revealed that it consisted of a 8881-bp *Eco*RI DNA fragment inserted into the *Eco*RI site of pUC18. Sequence analysis of the *EcoR1* fragment revealed eight open reading frames (ORFs), with a partial restriction map shown in Figure 1. The amino acid sequences of the predicted polypeptides from four of the ORFs show high homology to the amino acid sequences of polypeptides from other known three component dioxygenase systems, in particular the 2-nitrotoluene 2,3-dioxygenase enzyme system (2NTDOS) from *Pseudomonas* sp. strain JS42. Based on amino acid sequence analysis, four of the proteins were designated NBDR (reductase), NBDF (ferredoxin), $NBDO_{\alpha}$, and $NBDO_{\beta}$ (oxygenase) with gene designations *nbzAaAbAcAd*. Comparisons of NBDOS amino acid sequence homology are shown in table 1. Based on amino acid homology,

NBDOS is in the naphthalene/nitroaromatic dioxygenase family of enzymes. The reductase and ferredoxin components are identical in amino acid sequence to those in 2NTDOS, however $NBDO_{\alpha}$ and $NBDO_{\beta}$ are 95% identical. Also, a gene was found to encode a peptide with high homology to NahR, a regulatory protein involved in the regulation of the naphthalene degradation pathway genes (16, 19). Another ORF was found to be 99% identical to a similar ORF with no known function from the *ntd* operon of JS42 (9, 14). The seventh ORF encodes a protein with high homology to methyl-accepting chemotaxis proteins (MCP) from *E. coli*. The last ORF encodes a protein with homology to transposase (*Tn*) enzymes from *E. coli* and *Bacillus*.

Fig. 1. Partial restriction map of the 8.8-kb DNA fragment from *Comamonas* strain JS765



Nitrobenzene dioxygenase enzyme system

Conclusions. A gene cluster from *Comamonas* sp. strain JS765 encoding the nitrobenzene dioxygenase enzyme system was cloned and sequenced. NBDOS was found to be related to the 2-nitrotoluene dioxygenase enzyme system (2NTDOS) from *Pseudomonas* sp. strain JS42, 2,4-dinitrotoluene dioxygenase enzyme system (DNTDOS) from *Burkholderia* sp. strain DNT, and naphthalene dioxygenase enzyme system (NDOS) from *Pseudomonas* sp. strain NCIB 9816-4 (9, 20, 23). A possible regulatory gene, which may play a role in NBDOS regulation, was found upstream of the *nbz* gene cluster.

Table 1. NBDOS amino acid sequence comparisons

	% Amno acid identity with NBDOS component			
	Reductase	Ferredoxin	ISPα	ISPβ
2-Nitrotoluene Dioxygenase (JS42)	100	100	95	95
2,4-Ditrotoluene Dioxygenase (DNT)	91	79	87	96
Naphthalene Dioxygenase (U2)	99	83	88	92
Naphthalene Dioxygenase (9816-4)	67	72	82	78
Chlorobenzene Dioxygenase (P51)	21	35	36	25
Toluene Dioxygenase (PpF1)	21	36	36	26

2-Nitrotoluene dioxygenase from *Pseudomonas* sp. strain JS42, Parales et al., 1996; 2,4-Dinitrotoluene dioxygenase from *Pseudomonas* sp. strain DNT, Suen et al., 1995; Naphthalene dioxygenase from *Pseudomonas* sp strain U2, Fuenmayor et al., 1998; Naphthalene dioxygenase from *Pseudomonas* sp strain 9816-4, Simon et al., 1993, and Parales and Gibson, 1994; Chlorobenzene dioxygenase from *Pseudomonas* sp. strain P51, Werlen and van der Meer, 1994; Toluene dioxygenase from *Pseudomonas putida* F1, Zylstra and Gibson, 1989.

2) Characterization of NBDO.

Summary. NBDOS from JS765 is very similar in amino acid identity to other dioxygenase enzymes for which detailed substrate specificity studies have been performed (8, 10, 11, 13). It appears that the substrate specificity of Rieske non-heme iron dioxygenases lies in the C-terminal portion of the oxygenase α-subunit, based on mutagenesis studies and on the crystal structure of NDO (3, 8, 13). In order to compare the substrate specificity of NBDO to related enzymes, a recombinant *E. coli* strain expressing NBDOS from a plasmid was constructed. This recombinant strain was used to perform whole cell biotransformations with the compounds indicated below. Results revealed distinct differences in the substrate specificity of NBDO compared to 2NTDO, DNTDO, and NDO. NBDO was also purified to near homogeneity

Construction of recombinant *E. coli* strain for expression of NBDOS. Plasmid pDTG925 was digested with *Sac*I and a ~5-kb DNA fragment containing *nbzAaAbAcAd* was isolated and ligated into *Sac*I-digested pUC18 (24). Clones were screened for proper orientation so that *nbz* gene cluster is downstream of the *lac* promoter. A positive cloned was identified and named pDTG927. pDTG927 was transformed into *E. coli* DH5α. *E. coli* DH5α (pDTG927) was used for whole cell biotransformations.

Analysis of Substrate Specificity. The ability of NBDO to oxidize various substrates was determined. The substrates examined were nitrobenzene, 2, 3, and 4-nitrotoluene, and naphthalene. Whole cell biotransformations were performed using *E. coli* DH5α (pDTG927). Products were analyzed by thin-layer chromatography, gas-chromatography mass spectrometry (GC-MS), and chiral HPLC. Ratios of products formed as detected by GC-MS are compared to previously reported products formed by 2NTDO, DNTDO, and NDO shown in table 2. NBDO is able to oxidize naphthalene to naphthalene dihydrodiol with predominantly the (+)-(1*R*,2*S*)-enantiomer formed. The largest differences in regiospecificity of mono or dihydroxylation were noted with the isomers of nitrotoluene. Based on the substrates tested, NBDO appears to have an altered substrate specificity compared to the related dioxygenases, 2NTDO, DNTDO, and NDO. The difference in substrate specificity is probably due to different amino acid residues in the C-terminal portion of NBDO.

Table 2. Comparison of products formed by NBDO to closely related dioxygenase enzymes.

Substrate	NDO	2NTDO	DNTDO	NBDO
Naphthalene	>99% (+)-*cis*-(1*R*,2*S*)	70% (+)-*cis*-(1*R*,2*S*)	96% (+)-cis-(1*R*,2*S*)	65% (+)-*cis*-(1*R*,2*S*)
Nitrobenzene	None Detected	Trace	None Detected	
2-Nitrotoluene		90% 10%		55% 45%
3-Nitrotoluene		59% 27% 14%		
4-Nitrotoluene		76% 24%	89% 11%	

Purification of NBDO. *E. coli*DH5α (pDTG927) was used to facilitate the purification of NBDO. NBDO was purified to near homogeneity using ion-exchange and hydrophobic interaction chromatography with activity and yields shown in Table 3. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate revealed that NBDO is composed of a large (α) subunit and a small (β) subunit with an $\alpha_n\beta_n$ configuration (Fig. 2). The absorbance spectrum of oxidized NBDO showed maxima at 560 (shoulder), 462, and 330 nm, which are characteristic properties of Rieske iron-sulfur clusters (15). Oxidized NBDO was reduced in the presence of NADH and catalytic amounts of reductase and ferredoxin from the 2-nitrotoluene dioxygenase system (2NTDOS). Reduced NBDO gave an electron-paramagnetic resonance (EPR) spectrum with signals at $g_x = 1.75$, $g_y = 1.92$, and $g_z = 2.02$, again typical of Rieske iron-sulfur clusters. NBDO activity was demonstrated by measuring nitrite release from nitrobenzene in the presence of NADH, ferrous iron, and purified reductase and ferredoxin components from the 2-nitrotoluene dioxygenase system from *Pseudomonas* sp. strain JS42.

Table 3. Purification of Nitrobenzene Dioxygenase

Purification Step	Total protein (mg)	Activity (U*)	Specific Activity (U/mg protein)	Yield (%)
Crude cell extract	3750	80550	21.48	100
Q-Sepharose	315	49140	156.67	61
Butyl-Sepharose	15	9780	652.11	12

* U= nmoles NO_2^-/min. Activity was measured by release of nitrite from nitrobenzene by NBDO in the presence of NADH and catalytic amounts of $reductase_{2NT}$ and $ferredoxin_{2NT}$

Conclusions. Using a recombinant *E. coli* strain expressing NBDOS it was demonstrated that the substrate specificity of NBDO is different from the related dioxygenases, 2NTDO, DNTDO, and NDO. Purified NBDO was shown to be active as demonstrated by the nitrite release assay. Oxidized NBDO has an absorption spectrum typical of Rieske iron-sulfur proteins. Oxidized NBDO was reduced in the presence of NADH, $reductase_{2NT}$ and $ferredoxin_{2NT}$.

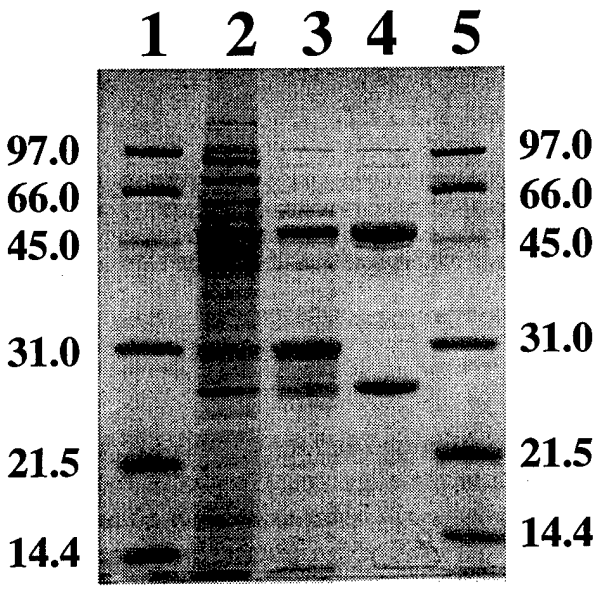


Fig. 2. SDS-PAGE analysis of samples taken during the purification of NBDO. Lanes 1 and 5: molecular weight standards (k-Da), lane 2: crude cell extract, lane 3: Q-sepharose, lane 4: butyl-sepharose.

3) Analysis of NBDOS gene expression.

Summary. Previous experiments with cell extracts indicated that expression of NBDOS in JS765 is inducible (7). In contrast, 2NTDOS expression appears to be constitutive, since a high level of cell extract activity was observed under all growth conditions (12). Interestingly, both of the proposed promoter sequences for the *nbz* and *ntd* operons and the putative regulatory proteins identified in each

strain are identical in both JS765 and JS42. Figure 3 shows a comparison of the proposed *nbz/ntd* promoters to the promoters for naphthalene degradation in *P. putida* G7. The high degree of similarity suggests a similar regulatory mechanism. It has been demonstrated in *P. putida* G7, which contains the Nah7 plasmid for naphthalene degradation, that salicylate induces expression of the naphthalene degradation genes and that NahR is required for induction (18, 19). The regulatory proteins identified in JS765 and JS42 are 61% identical to NahR from G7. Gene reporter technology was used to determine if the regulation of NBDOS and 2NTDOS is at the transcriptional level and what inducers stimulate gene expression.

```
                ** ****** * ** ****        *      ++ + +++      +++          +   +   +
JS765     ATGCCTCACCATTATTCATGCTGGTGATTTTAACTATCAGACTTGATCTATAGCGCTATACCGATCGACGCGCCAGTATCCG
JS42      ATGCCTCACCATTATTCATGCTGGTGATTTTAACTATCAGACTTGATCTATAGCGCTATACCGATCGACGCGCCAGTATCCG
G7sal     GGCCGGGCGCAATATTCATGTTGATGATTTATTATATATCGAGTGGTGTATTTATCAATATTGTTTGCTCCGTTATCGTTAT
G7nah     GACGATTCGCAGTATTCACGCTGGTGATAAACAAATTCAACTATGCTTTATTGACAAATAAAAGCACGCTCACCATCATCGC
                                                          -35                  -10
Consensus          CGCA-TATTCA-G-TG-TGAT--A--A--T
NahR binding
```

Fig. 3. Sequence alignment of the naphthalene and nitroarene dioxygenase promoters. A consensus NahR binding sequence is shown below the alignment. Nucleotides in the NahR binding region that are conserved in all promoters are indicated by asterisks. Other conserved nucleotides in the region are indicated by plus signs. The -35 and -10 sites of the *sal* and *nah* promoters from *P. putida* G7 are underlined (17). Possible nitroarene dioxygenase -35 and -10 sites that correspond to the G7 sites are also underlined.

Construction of *nbz/ntd-lacZ* reporter strain. To analyze transcription of the *nbz* and *ntd* gene clusters the promoters of each operon were fused to the *lacZ* gene, which encodes β-galactosidase . A 240-bp PCR-generated DNA fragment containing the putative promoter and NahR binding site was ligated upstream of *lacZ* in the vector pTL61T to form pDTG930. *Not*I-digested pDTG930 was ligated to *Not*I-digested pUTminiTn5-Gm to yield pDTG933. pDTG933 is a mobilizable vector containing an *nbz/ntd-lacZ* transcriptional fusion and a gentamicin resistance marker within the mini-Tn5. Introduction of pDTG933 into JS765 and JS42 was done by mating with *E. coli* S17-1 (pDTG933). Four transconjugants for both JS765 and JS42 that contained single Tn5 insertions at locations other than the *nbz* or *ntd* gene clusters and that showed growth on the respective nitroaromatic that was indistinguishable from that of wild-type were used for expression studies

Analysis of *lacZ* expression in JS765 and JS42. To analyze *lacZ* expression in reporter constructs, cultures were grown in minimal media supplemented with succinate alone, succinate + salicylate, or succinate + nitroaromatic substrates. β-Galactosidase activity was measured during logarithmic growth as outlined by Miller (5). Preliminary expression results are shown in Figure 4. It appears that both the *nbz* and *ntd* operons are inducible by their respective nitroaromatic substrates and salicylate. There is approximately a10-fold induction of the *nbz* operon with nitrobenzene and 40-fold with salicylate in JS765. In JS42 there is approximately a 5-fold induction with 2-nitrotoluene and 6-fold induction with salicylate. However, the background level of expression is much higher in JS42, roughly equal to the induced level in JS765. These results indicate that *nbz* and *ntd* gene clusters in JS765 and JS42, respectively are induced in the presence of the nitroaromatic compound and salicylate.

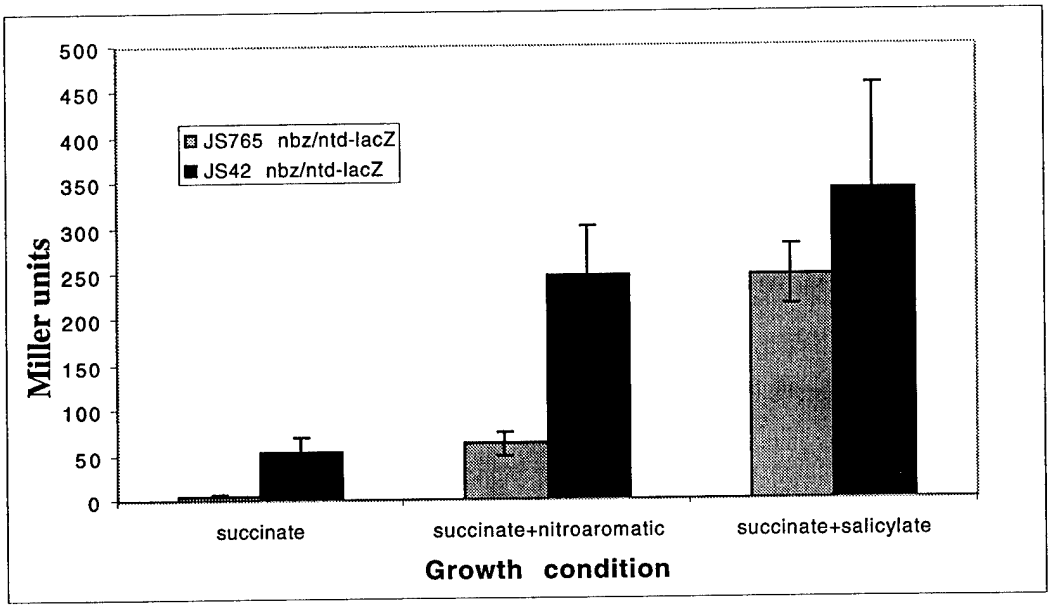


Table 4. *nbz.ntd-lacZ* fusion activities in JS765 and JS42 under non-inducing and inducing conditions.

Conclusions. Analysis of the gene reporter fusion suggests that both the NBDOS and 2NTDOS gene clusters are inducible in JS765 and JS42, respectively. However, it appears that the basal level of expression of 2NTDOS is much higher in JS42. This difference in basal expression could be due to differences in intrinsic promoter strength in JS765 and JS42. Also, salicylate was shown to be an inducer in both strains, suggesting that the regulatory machinery utilized for degradation of nitroaromatic compounds may have evolved from that of a naphthalene degrading organism.

References

1.**Haigler, B. E., W. H. Wallace, and J. C. Spain.** 1994. Biodegradation of 2-nitrotoluene by *Pseudomonas* sp. strain JS42. Appl. Environ. Microbiol. **60:**3466-3469.

2.**Hartter, D. R.** 1985. The use and importance of nitroaromatic chemicals in the chemical industry, p. 1-14. *In* D. E. Rickert (ed.), Toxicity of nitroaromatic compounds. Chemical Industry Institute of Toxicology Series. Hemisphere, Washington, D.C.

3.**Kauppi, B., K. Lee, E. Carredano, R. E. Parales, D. T. Gibson, H. Eklund, and S. Ramaswamy.** 1998. Structure of an aromatic ring-hydroxylating dioxygenase- naphthalene 1,2-dioxygenase. Structure. **6:**571-586.

4.**Keith, L. H., and W. A. Telliard.** 1979. Priority pollutants I-a perspective view. Environ. Sci. Technol. **13:**416-423.

5.**Miller, J. H.** 1975. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor , N.Y.

6.**Nishino, S. F., G. C. Paoli, and J. C. Spain.** 2000. Aerobic degradation of dinitrotoluenes and the pathway for bacterial degradation of 2,6-dinitrotoluene. Appl. Environ. Microbiol. **In press.**

7.**Nishino, S. F., and J. C. Spain.** 1995. Oxidative pathway for the biodegradation of nitrobenzene by *Comamonas* sp. strain JS765. Appl. Environ. Microbiol. **61:**2308-2313.

8.**Parales, J. V., and D. T. Gibson.** 1999. Identification of single amino acid changes that alter the substrate specificity and enantioselectivity of 2-nitrotoluene 2,3-dioxygenase. Q-249, p. 579. *In*

Abstracts of the 99th General Meeting of the American Society for Microbiology 1999. American Society for Microbiology.

9.**Parales, J. V., A. Kumar, R. E. Parales, and D. T. Gibson.** 1996. Cloning and sequencing of the genes encoding 2-nitrotoluene dioxygenase from *Pseudomonas* sp. JS42. Gene. **181:**57-61.

10.**Parales, J. V., R. E. Parales, S. M. Resnick, and D. T. Gibson.** 1998. Enzyme specificity of 2-nitrotoluene 2,3-dioxygenase from *Pseudomonas* sp. strain JS42 is determined by the C-terminal region of the α subunit of the oxygenase component. J. Bacteriol. **180:**1194-1199.

11.**Parales, R. E., M. D. Emig, N. A. Lynch, and D. T. Gibson.** 1998. Substrate specificities of hybrid naphthalene and 2,4-dinitrotoluene dioxygenase enzyme systems. J. Bacteriol. **180:**2337-2344.

12.**Parales, R. E., and D. T. Gibson.** Unpublished data. .

13.**Parales, R. E., K. Lee, S. M. Resnick, H. Jiang, D. J. Lessner, and D. T. Gibson.** 2000. Substrate specificity of naphthalene dioxygenase: effect of specific amino acids at the active site of the enzyme. J. Bacteriol. **182:**1641-1649.

14.**Parales, R. E., J. V. Parales, R. An, A. Kumar, S. M. Resnick, J. C. Spain, and D. T. Gibson.** 1995. 2-Nitrotoluene dioxygenase from *Pseudomonas* sp. strain JS42: protein purification, molecular biology and substrate specificity. D-12, p. 95 *In:* Abstracts, *Pseudomonas* 1995, Fifth International Symposium on *Pseudomonas*: Biotechnology and Molecular Biology.

15.**Rieske, J. S., D. H. Maclennan, and R. Coleman.** 1964. Isolation and properties of an iron-protein from the (reduced coenzyme Q)-cytochrome C reductase complex of the respiratory chain. Biochem. Biophys. Res. Commun. **15:**338-344.

16.**Schell, M. A.** 1983. Cloning and expression in *Escherichia coli* of the naphthalene degradation genes from plasmid NAH7. J. Bacteriol. **153:**822.

17.**Schell, M. A.** 1986. Homology between nucleotide sequences of promoter regions of *nah* and *sal* operons of NAH7 plasmid of *Pseudomonas putida*. Proc. Natl. Acad. Sci. USA. **83:**369-373.

18.**Schell, M. A.** 1990. Regulation of naphthalene degradation genes of plasmid NAH7: example of a generalized positive control system in *Pseudomonas* and related bacteria, p. 165-177. *In* A. M. Chakrabarty, S. Kaplan, B. Iglewski, and S. Silver (ed.), Pseudomonas: Biotransformations, pathogenesis, and evolving biotechnology. American Society for Microbiology, Washington, D.C.

19.**Schell, M. A., and E. F. Poser.** 1989. Demostration, characterization, and mutational analysis of NahR protein binding to *nah* and *sal* promoters. J. Bacteriol. **171:**837-846.

20.**Simon, M. J., T. D. Osslund, R. Saunders, B. D. Ensley, S. Suggs, A. Harcourt, W.-C. Suen, D. L. Cruden, D. T. Gibson, and G. J. Zylstra.** 1993. Sequences of genes encoding naphthalene dioxygenase in *Pseudomonas putida* strains G7 and NCIB 9816-4. Gene. **127:**31-37.

21.**Spain, J. C.** 1995. Bacterial biodegradation of nitroaromatic compounds under aerobic conditions, p. 19-35. *In* S. J.C. (ed.), Biodegradation of nitroaromatic compounds, vol. 49. Plenum Press, New York.

22.**Spain, J. C.** 1995. Biodegradation of nitroaromatic compounds. Annu. Rev. Microbiol. **49:**523-555.

23.**Suen, W.-C., B. E. Haigler, and J. C. Spain.** 1996. 2,4-Dinitrotoluene dioxygenase from *Burkholderi*a sp. strain DNT: Similarity to naphthalene dioxygenase. J. Bacteriol. **178:**4926-4934.

24.**Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene. **33:**103-119.

PERSONNEL SUPPORTED

Daniel J. Lessner

PUBLICATIONS

1. Lessner, D.J., G.R. Johnson, R.E. Parales, J.C. Spain, and D.T. Gibson. **Cloning and characterization of nitrobenzene dioxygenase from *Comamonas* sp. strain JS765.** (In preparation).

PRESENTATIONS

1. Daniel J. Lessner presented a poster, "**Cloning and nucleotide sequence of the genes encoding nitrobenzene dioxygenase from *Comamonas* sp. strain JS765**" at the 8th Biocatalysis and Bioprocessing Conference, University of Iowa. October, 1999.

2. Daniel J. Lessner presented a poster, "**Cloning and characterization of the genes encoding nitrobenzene dioxygenase from *Comamonas* sp. strain JS765**" at the 99th General Meeting of the American Society for Microbiology, Los Angeles, Ca., May 2000.

3. Daniel J. Lessner presented a poster, "**Purification and characterization of nitrobenzene dioxygenase from *Comamonas* sp. strain JS765**" at the 9th Biocatalysis and Bioprocessing Conference, University of Iowa. October, 2000.